Three-dimensional (3D) culture of bone-derived human 786-O renal cell carcinoma retains relevant clinical characteristics of bone metastases

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Background:

Bone metastases from renal cell carcinoma (RCC) are typically lytic, destructive, and resistant to treatment regimens. Current in vitro models for studying metastasis introduce artifacts that limit their usefulness. Many features of tumors growing in bone are lost when human RCC cells are cultured in two-dimensional (2D) on plastic substrata. 3D hydrogels composed of hyaluronan or modified hyaluronan have been previously shown to support the long term growth of bone metastatic PCa cells, including those from patient-derived xenografts. In this study, we examined whether 3D cultures of the human 786-O RCC subline derived from bone metastases in HA-based hydrogel would provide a useful model for studying treatment resistance in RCC.

Questions/Purposes:

1. Whether RCC cells grown in 3D system retain a phenotype and express biomarkers that mimic the tumors in vivo?
2. Whether 786-O RCC cells in 3D express molecules that promote angiogenesis or osteolysis that are characteristics of RCC bone metastases?

Patients and Methods:

Bone-derived 786-O RCC cells (bone-786-O RCC) used in this study were generated from bone metastases from a patient with renal clear cell carcinoma. Cells were cultured at 37°C with 5% CO2 in RPMI medium (Invitrogen) supplemented with 10% (v/v) fetal bovine serum (FBS). Thiol-modified HA and extralink poly ethylene glycol-diacrylate (BioTime Inc. Alameda, CA) were used for encapsulating cells according to the manufacturer's instructions. The hydrogel constructs were then incubated at 37°C for 30 min to allow for polymerization. Complete medium was then added to fully submerge the hydrogel constructs and incubated overnight. The next day, the hydrogel constructs were transferred to wells of 48-well plates containing 500 µl of complete medium in each well. Culture medium was changed every other day. The PrestoBlue reagent kit (Life Technologies, Grand Island, NY) was used to measure cell viability over time. To visualize cell viability, cells were stained with Live/Dead viability/cytotoxicity assay kit and then imaged using a Leica SP5 CLSM confocal microscope. Total RNA was extracted from cells using the RNeasy mini purification kit and single-strand cDNA was synthesized using the TaqMan Reverse Transcription Reagents. Real-time PCR was performed using the SYBR-Green PCR master mix (Invitrogen) on a Multiplex Quantitative PCR System. Protein levels of vascular endothelial growth factor (VEGF) and receptor activator of nuclear factor kappa-B ligand (RANKL) in cell culture medium were determined using a human VEGF ELISA kit and a human RANKL ELISA kit respectively. The absorption OD value was measured at 450 nm.

Results:

In this study, we established that RCC spheroids, consisting of aggregates of cells, can be grown in a three-dimensional (3D) hyaluronate hydrogel-based culture system. The bone-derived human 786-O RCC subline proliferated and survived long term in these hydrogels. Additionally, RCC spheroids in 3D hydrogels demonstrated lower proliferation rates than their counterparts grown in 2D. Of particular importance, selected adhesion molecules, angiogenesis factors and osteolytic factors that have been shown to be involved in RCC bone metastasis were found to be expressed at higher levels in 3D than in 2D cultures, indicating that the gene expression patterns of RCC spheroids in 3D more closely mimicked those observed *in vivo* than did those of cells grown in 2D.

Conclusions:

In summary, bone-derived 786-O RCC cells growing in 3D HA-based hydrogels form spheroids that are held together by adhesion molecules common to solid tumors. In contrast to 2D, they express genes that model

the in vivo characteristics of RCC bone metastasis including those gene products associated with hypervascularity and osteolysis. We propose to better characterize the phenotype of the RCC spheroids and to determine their suitability for pharmaceutical testing. The 3D model also provides a potentially higher fidelity means to study the mechanism of metastasis formation compared to 2D culture; and for identifying interventions strategies targeting the growth of metastases in bone.